that the IVa2 and L4-22K proteins specifically bind to conserved elements within the PS, and that these interactions are absolutely required to turn on the virus assembly pathway. The IVa2 and L4-22K proteins, along with an alternatively spliced product of the L4-22K gene, called L4-33K, also function as transcriptional activators of the major late promoter (MLP). The MLP primarily controls production of viral structural proteins for eventual construction of viral particles. IVa2, L4-22K and L4-33K activate transcription of the MLP by binding to DNA sequences downstream of the MLP (called DE), which are composed of the identical sequences found within the PS. The timing of the onset of the virus assembly pathway is critical to ensure optimal viral production; if virus assembly is initiated prematurely, production of viral components will be suppressed leading to poor viral production. Thus, a critical question is how is it possible to simultaneously activate transcription from the MLP while ensuring viral assembly is not initiated prematurely, using an identical set of control proteins and conserved DNA sequences? A fundamental difference between the PS and the DE is that the DE resembles a single functional DNA binding sequence, while the PS possesses multiple binding sequences. Thus, we propose cooperative interactions between the viral control proteins serve to switch on virus assembly. We are testing this hypothesis by constructing a physical-chemical model to describe quantitatively this switching behavior.

RNA Folding

3267-Pos Board B128
Nanomanipulation of Single RNA Molecules
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An RNA sequence can fold into many thermodynamically stable structures on a drawing board. However, experimentalists focus on RNAs dominated by a single most stable structure because it is difficult to distinguish polymorphic structures, and because it is impossible to have many molecules to uniformly adopt a suboptimal structure. Many experiments are done on folding trajectories directly leading to the most stable conformations. Such a narrow folding energy landscape excludes potential interesting observations, including alternative conformations, intermediates, and routes. Many of these alternative folding are employed in gene regulations, such as transcription attenuation and riboswitch. A solution to survey manifold folding energy landscape is single-molecule nanomanipulation technique using optical tweezers. Examining one molecule at a time eliminates necessity to deconvolute structure polymorphism adopted by many molecules, a major challenge in ensemble studies. Structure manipulation with nanometer precision allows access and identification of suboptimal or even rare structures and folding pathways. Specifically, an RNA molecule is stretched and relaxed from its 5'- and 3'-ends like a rubber band. Applied force destabilizes structures to various degrees depending on folding energy and size of each structure. Therefore, modulation of force changes structure populations of an RNA. We will illustrate this idea by two examples. An RNA kissing complex is manipulated into multiple stable conformations, each with a distinctive end-to-end distance. This method makes it possible to access folding intermediates and measure substep kinetics. In another example, a single-strand is manipulated into various stable and metastable structures, and a subsequent force protocol lead these structure to the most stable conformation.

3268-Pos Board B129
Determining Molecular Structure Using Nanopores
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RNA molecules contain sequence-dependent secondary structures that yield specific biological functions.
One approach to study RNA structure employs optical tweezers in which individual RNA molecules, tethered between micrometer-sized particles, are progressively pulled apart.

Since folded regions unzip in order of increasing stability, the shortcoming of this approach is that the readout does not yield the location of the unfolding regions, rendering reconstruction of the secondary structure cumbersome if not impossible. Another approach is pulling RNA molecules through nanopores, which reports on molecular structure through parameters as current blockade and translocation time.
We propose to integrate these two approaches to measure the secondary structure of RNA. This integrated approach allows one to sequentially unfold the molecule and simultaneously measure the position and stability of the unfolding regions. As a proof of concept, we will measure the unfolding of a single RNA hairpin as shown in the adjacent figure. This new approach fills the gap in currently available techniques and provides a more straightforward determination of molecular secondary (and potentially tertiary) structure. Results of such measurements will be an important step forward in solving the outstanding RNA folding problem and understanding the shape-dependent function of RNA molecules.

3269-Pos Board B130
RNA Flexibility and Folding in Crowded Solutions
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RNA molecules must fold to form their biologically active native state in the crowded environment of cells. Most folding experiments, however, are carried out in dilute solution, and so require corrections to their outcomes to be applicable in vivo. We are studying RNA structure and folding in crowded solutions to empirically determine principles for such corrections, and therefore determine the true stability of folded RNA molecules in the cellular milieu.
We used small angle x-ray scattering experiments to measure scattering functions of a 64 kDa bacterial group I ribozyme in the presence of stabilizing ions (MgCl2 and NaCl) and polyethylene-glycol with different molecular weights as crowders.
We find that crowder molecules favor more compact states of the unfolded RNA, and also stabilize the folded state with respect to the unfolded state by up to 5 kT, as measured via the lowering of the folding midpoint on both MgCl2 and NaCl titrations. Crowders are also observed to compress the native state of this ribozyme and we have measured a concurrent increase in ribozyme activity. These effects cannot be explained by changes in Mg2+ or water activity.
In addition, we compare the scattering functions of RNAs stabilized to the same Rg in solutions with different crowders. We observe lower intensity scattering at approx. $Q > 0.08$ Å-1 for RNA in higher ionic strength solutions, indicating lower electron density correlations within those RNA particles on length scales below 80 Å. From the scattering functions we calculate the correlation length per volume for the particles and show that crowders restrict the number of conformations accessible to the RNA molecule compared with RNA stabilized exclusively by ions.

3270-Pos Board B131
Single Molecule Analysis of Human Telomerase RNA Pseudoknot Folding and Dynamics
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The essential telomerase ribonucleoprotein (RNP) enzyme adds telomere DNA repeats to chromosome ends, combating chromosome degradation and erroneous DNA repair processes. The telomerase RNP is minimally comprised of the telomerase reverse transcriptase protein (hTERT) and telomerase RNA (hTR). hTR provides the template sequence for the reverse transcription reaction and serves as a scaffold for other telomerase-associated proteins. Among several conserved structural motifs within hTR, the structure of a pseudoknot fold that undergoes compaction into a triple helical structure has been solved using NMR. We established a single-molecule FRET based assay to characterize the structural dynamics of the hTR pseudoknot in the context of the native RNA sequence. The effects of mutations affecting pseudoknot or triplex stability have been studied in both minimal and native RNA constructs. Our results suggest the folding properties of the hTR pseudoknot are significantly influenced by the presence of proximal RNA structural elements, affecting pseudoknot formation in the functional hTR sequence. We also demonstrate that the structural manifestation of a specific pathogenic mutation within the hTR pseudoknot depends on both the sequence context and Mg(II) concentration. The energetic stabilization conferred by (i) the native pseudoknot structure, (ii) the binding of Mg(II) ions, and (iii) the triplex structure all contribute individually to the folding of the functional hTR. Importantly, in the absence of any one of these three stabilizing factors the native hTR sequence is unable to successfully navigate the rugged energy landscape toward its native fold.

3271-Pos Board B132
Mechanism of Enhanced Mechanical Stability of Retroviral Kissing-Loops Elucidated by Non-Equilibrium Molecular Dynamics Simulations
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